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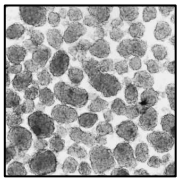

Cells on Demand™ Fresh Human Pancreatic Islets

Technical Information & Instructions

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I. Introduction

Cells on Demand™ Fresh Human Pancreatic Islets (or Islets of Langerhans) contains Human Pancreatic Islets isolated from fresh donor tissue and media for maintenance of cultures prior to and during experimental procedures. Human Pancreatic Islets may be used for studies of long-term islet grafting survival, prevention of islet rejection, prevention of adverse effects from immunosuppressive drugs and basic research. Fresh Human Pancreatic Islets are shipped in primary passage.

II. Performance

Fresh Human Pancreatic Islets are non-proliferating, suspension cell aggregates and can remain optimally viable in culture at 22℃ for approximately seven d ays from the date of isolation, but decline in viable number over time. Cultures longer than seven days (possibly up to 4 weeks) may be possible for specific applications with frequent media changes and special culture conditions. See references for more information. For optimal viability, use cells immediately upon receipt, but allow the islets to equilibrate to assay conditions (i.e. 37℃, different media) for 12-24 hours for proper physiological function.

III. Culturing References

1. Daoud J, Rosenberg L, Tabrizian M. Pancreatic islet culture and preservation strategies: advances, challenges, and future outlook. Cell Transplant. 2010;19(12):1523-35.
2. Kerr-Conte J, Vandewalle B, Moerman E, Lukowiak B, Gmyr V, Arnalsteen L, Caiazzo R, Sterkers A, Hubert T, Vantyghem MC, Pattou F. Upgrading pretransplant human islet culture technology requires human serum combined with media renewal. Transplantation. 2010 May 15;89(9):1154-60.
3. Murdoch TB, McGhee-Wilson D, Shapiro AM, Lakey JR. Methods of human islet culture for transplantation. ll Transplant. 2004;13(6):605-17.

IV. General Cell Information

Description	Recommended Culture Media	Passage Number	Recommended Culturing Density
Fresh Human Pancreatic Islets, 100 K	(Included with order)	Passage 0	500-1000 IEQ/ml
Fresh Human Pancreatic Islets, 20 K	(Included with order)	Passage 0	500-1000 IEQ/ml
Fresh Human Pancreatic Islets, 10 K	(Included with order)	Passage 0	500-1000 IEQ/ml
Fresh Human Pancreatic Islets, 5 K	(Included with order)	Passage 0	50-500 IEQ/ml
Fresh Human Pancreatic Islets, 2 K	(Included with order)	Passage 0	50-500 IEQ/ml

V. Quality Control

All cells test negative for HIV-1, Hepatitis-B, Hepatitis-C, and bacteria. Certificates of Analysis (COA) for each cell strain are shipped with each order that include IEQ count, %purity and %viability as indicated by DTZ staining. IEQ's must have a viability at time of shipping of ≥70% and purity of ≥70%. Please note that one islet equivalent (IEQ) is an islet of diameter 150 µm, typically containing 1,500-2,000 cells, including 40-60% beta cells. Islets of different sizes are volumetrically adjusted to IEQs during enumeration.

VI. Unpacking and Storage Instructions

1. Upon receiving the shipping box, inspect the box for mishandling/damage during shipment. Document any abnormality.
2. Open the box carefully and make note of the following:
    a. HOBO® Temperature Monitor*
    b. Return Label Shipment Pouch
    c. Pancreatic Islet Shipping Vessel
    d. Pancreatic Islet Media Bottle(s)

    *Located near the islet shipping vessel. Document location if otherwise.
3. Check the islet shipping vessel and media bottle for leakage or breakage. Document condition if necessary.
4. Islet Culture & Media Instructions: store Pancreatic Islet Media at 2°-8℃. Use media within seven days of the date of receipt. Do not freeze.
5. Remove the pancreatic islet shipping vessel from shipping box.
6. Ensure the HOBO® Temperature Monitor device remains in the shipping box and is not transferred to the incubator with the shipping vessel.
7. Swab down the pancreatic islet shipping vessel with 70% ethanol or isopropanol, then place the vessel in a 22℃, 5% CO_2, humidified incubator and allow to equilibrate for three to four hours. After cells have equilibrated, remove the excess shipping media and change the remaining media as described in the Initiation of Culture Process & Maintenance section below.
8. Return the HOBO® Temperature Monitor and shipping gel packs as soon as possible in the original shipment box by using the return label.
9. Dispose of any absorbent pads that are wet or soiled. Remove the shipping label from the original shipping box and replace with the prepared Return Label shipment pouch. Return the gel pack temperature stabilizers and packing material if possible.

VII. Initiation of Culture Process & Maintenance

NOTE: Fresh Human Pancreatic Islets are shipped in a Wilson Wolf G-Rex® gas permeable cell culture device. Islets may be maintained in the original shipping vessel or transferred to alternate vessels upon receipt. Please see Section III (Culturing References, Page 1) for suggestions on alternate culturing vessels and conditions.

1. Upon receipt, islets shipped in a Wilson Wolf G-Rex®100 vessel will be suspended in ~500 mL of Islet Culture & Shipping Media. Islets shipped in a Wilson Wolf G-Rex®10 vessel will be suspended in ~40 mL of Islet Culture & Shipping Media
2. Remove excess culture media and change ~50% of the remaining media upon receipt, and change ~50% of the culture media every three to four days thereafter (allow islets to settle by gravity 3-5 minutes before removal):

- Islets shipped in a Wilson Wolf G-Rex®100 vessel (20K to 200K IEQ products) should be maintained in ≥200 mL of media
- Islets shipped in a Wilson Wolf G-Rex®10 vessel (2K to 10K IEQ products) should be maintained in ≥35 mL of media

3. Warm an appropriate amount of medium to 20°-24℃ in a sterile container.
4. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.
5. In a sterile field, securely place the islet shipping vessel at ~20° angle and allow the islets to settle for 3-5 minutes.
6. Slowly pipette off all of the supernatant except ~100 mL in the G-Rex®100 vessel or ~7.5 mL in the G-Rex®10 vessel and place the supernatant in a sterile container. While pipetting, avoid submerging the pipette tip to the bottom of the vessel as doing so may result in the loss of islets or possible puncture to the oxygen permeable silicone rubber membrane on the bottom of the vessel.
7. Transfer ~20 mL of the supernatant to a T-75 flask and examine the supernatant microscopically. If no islets are present, properly dispose of the supernatant. If a significant number of islets is present in the supernatant, return the supernatant to the culture vessel and repeat steps 5 through 7.
8. If sampling islets or transferring to an alternate vessel, gently rock the culture vessel to ensure uniform islet suspension. Remove the necessary volume of islet suspension using a ***wide orifice pipette tip*** and transfer to an appropriate vessel. Please see Section VIII (Counting Islets & Determining Purity Using Dithizone, Page 4) for more information on counting islets.

NOTE: Fresh Human Pancreatic Islets may adhere to the silicone rubber membrane on the bottom of the culture vessel. To remove islets, wash liberally with culture media.

9. When media change or sampling is complete, replenish the culture media to the recommended volume. Place the culture vessels into a 22°C humidified incubator with 5% CO_2.

NOTE: Islets should be cultured at 37°C for >12 hours for functional assays to achieve physiological conditions. Culture at 22°C is more appropriate for maintenance of islet number. These cells cannot be subcultured or cryopreserved.

VIII. Counting Islets & Determining Purity Using Dithizone

Pancreatic islets consist of a cluster of various hormone-producing cells. As each cluster contains various quantities of cells, the actual number of islets present (AI) is not necessarily representative of the number of cells present. Islets of different sizes can be volumetrically adjusted to Islet Equivalents (IEQ) during enumeration to provide a more accurate description of the number of cells present. One islet equivalent (IEQ) is an islet of diameter 150 µm, typically containing 1,500-2,000 cells, including 40-60% beta cells. When counting islets, islets can be distinguished from non-islet tissue by using Dithizone (DTZ) to stain red the zinc granules in the beta cells. The percent purity can be presented as the percentage of islets compared to all tissue present in the islet preparation (islets, acinar and ductal cells), determined by visual inspection of a representative sample of the islet preparation.

Required Components (Sold Separately)

- One Fresh Human Pancreatic Islets product – (fresh)
- Dulbecco's Phosphate Buffered Saline (DPBS) (1X); 9.5 mM (PO_4) with calcium and 0.0095M (PO_4) magnesium - 500 mL (Lonza Catalog No. 17-513F, or similar)
- Dithizone (DTZ) (Sigma Catalog No. D5130, or similar)
- Dimethyl Sulfoxide (DMSO) (Sigma Catalog No. 34869, or similar)
- Sterile 10 x 35 mm counting dishes with grid marks (Nunc Catalog No. 174926, or similar)
- Nylon Filter with 0.45 µm Pore Size (Sigma Catalog No. 58067, or similar)
- Light Microscope with Calibrated Eyepiece Reticle with Divisions of 0.1 mm

NOTE: For statistical accuracy and relevance, it is suggested that the following protocol be performed in triplicate and the results averaged.

1. Formulate 1 mg/mL Dithizone (DTZ) Stock Solution by dissolving 5 mg Dithizone (DTZ) in 1

mL Dimethyl Sulfoxide (DMSO) in a sterile 15 mL conical tube. Thoroughly mix the solution, then add Dulbecco's Phosphate Buffered Saline (DPBS) to a final volume of 5 ml. Filter the combined solution through a nylon filter with 0.45 µm pore size into a sterile 15 mL conical tube. Use within 24 hours.

2. Prepare islet suspension for counting by gently but thoroughly mixing the suspension by inverting the suspension in a conical tube 2-3 times prior to sampling. Do not swirl.

NOTE: Fresh Human Pancreatic Islets may settle quickly. Care must be taken to ensure a representative sample is taken.

3. Using a wide orifice pipette tip, transfer 100 µL of the islet suspension to the center of a sterile 10 x 35 mm counting dishes with grid marks allowing the solution to form a droplet.
4. Add 30 µL of 1 mg/mL Dithizone (DTZ) Stock Solution into the islet suspension droplet and mix gently. Allow staining for 1-2 minutes at room temperature.
5. Cover the bottom of the counting dish with DPBS to approximately ½ the height of the dish (approximately 1 mL of DPBS). Rock the plate gently to evenly distribute islets.
6. Using a light microscope with a calibrated eyepiece reticle with divisions of 0.1 mm set on the 4X objective and using the grid lines on the counting dish as a guide, methodically scroll through the dish from side to side, and top to bottom, examining each islet. Count islets within the perimeter of the grid's squares, including only islets touching the top and right lines (not the bottom and left lines) to avoid counting the same islet twice. DTZ stained islets will appear red.
7. Using an eyepiece reticle with divisions of 0.1 mm set on the 4X objective, the distance across two divisions on the calibrated reticle in the eyepiece will equal 50 µm. It is not necessary to count islets smaller than 50 µm as their contribution is not significant. Using Table 1 (Guide for Counting Islets and Determining Islet Purity, Page 5) as a guide, record the number of islets in each of the diameter groups. When determining purity, also record the number of non-islet in the appropriate area on Table 1.

NOTE: The magnification of the eyepiece is irrelevant when utilizing a reticle. Alternate reticles and magnifications may be utilized so long as they are calibrated correctly to measure in increments of no more than 50 µm and total sizes of up to 350 µm. Additionally, programmable cell counters capable of measuring cells by size may be used in place of manual counting methods.

8. Determine the dilution factor by using the following equation and enter this value into the appropriate section in Table 1:

$$\text{Dilution Factor} = \frac{\text{Total Volume of Islet Suspension (ml)}}{\text{Volume of Sample (µl)}} \text{x} 1000$$

9. Determine the number of actual islets (AI) present in the sample by adding the quantity of actual islets present for each islet diameter range. Enter this total into the appropriate section in Table 1.

Table 1. Guide for Counting Islets and Determining Islet Purity

Number of Divisions Spanned	Islet Diameter Rang (µm)	Actual Islets Present (AI)		IEQ Conversion Factor		IEQ
2-4	50-100		x	0.167	=	
4-6	101-150		x	0.648	=	
6-8	151-200		x	1.685	=	
8-10	201-250		x	3.500	=	
10-12	251-300		x	6.315	=	
12-14	301-350		x	10.352	=	
>14	>350		x	15.833	=	
Islets in Sample (ΣAI) =				*IEQ in Sample* (ΣIEQ) =		
Number of Non-Islets (non-red, acinar and ductal cells) =						
Percent Purity: (ΣAI / [ΣAI + Number of Non-Islets]) x 100% =						
Dilution Factor: (ml total volume / µL sample volume) x 1000 =						
Total AI: ΣAI x Dilution Factor =						
Total IEQ: ΣIEQ x Dilution Factor =						

10. Determine the total number of actual islets (AI) present in the total volume of islet suspension by multiplying the Islets in Sample (ΣAI) by the Dilution Factor. Enter this total into the appropriate section in Table 1.
11. Determine the percent purity by using the following equation and enter this value into the appropriate section in Table 1:

$$\text{Percent Purity} = \frac{\text{Islets in Sample } (\Sigma AI)}{\text{Islets in Sample } (\Sigma AI) + \text{Number of Non - Islets}} \times 100\%$$

12. Multiple the quantity of actual islets (AI) present for each islet diameter range by the corresponding Islet Equivalents (IEQ) Conversion Factor to obtain the IEQ value for each diameter range. Enter this converted value for each diameter range into the appropriate section in Table 1.
13. Determine the IEQ present in the sample by adding the quantity of IEQ for each islet diameter range. Enter this total into the appropriate section in Table 1.
14. Determine the total IEQ present in the total volume of islet suspension by multiplying the IEQ present in Sample (ΣIEQ) by the Dilution Factor. Enter this total into the appropriate section in Table 1

IX. Determining Glucose Stimulated Insulin Response

Insulin production is a critical function of the beta cells found within pancreatic islets. Insulin plays a critical role in the regulation of metabolism, carbohydrates, and fat. As such, an important measurement in pancreatic islets is determining insulin response as triggered by insulin. The Glucose Stimulated Insulin Response (GSIR) assay measures the ability of pancreatic islets to produce insulin when stimulated by an increase in the concentration of glucose. Functional assessment of purified human pancreatic islets: glucose stimulated insulin release by ELISA – A.

Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium; CellR4 2014; 2 (2): e900

Required Components (Sold Separately)

- One Fresh Human Pancreatic Islets product and Culture Medium – (fresh)
- HEPES powder (Sigma Catalog No. H4034, or similar)
- Sodium Chloride (NaCL) (Sigma Catalog No. S7653, or similar)
- Sodium Bicarbonate ($NaHCO_3$) (Sigma Catalog No. S6297, or similar)
- Potassium Chloride (KCl) (Sigma Catalog No. P9333, or similar)
- Magnesium Chloride Hexahydrate ($MgCl_2$-$6H_2O$) (Sigma Catalog No. M2670, or similar)
- Calcium Chloride Dihydrate ($CaCl_2$-$2H_2O$) (Sigma Catalog No. C5080, or similar)
- Bovine Serum Albumin (BSA) (Sigma Catalog No. A2153, or similar)
- D-(+)-Glucose (Dextrose) (Sigma Catalog No. G7528, or similar)
- 1.0N Sodium Hydroxide (NaOH) Solution (Sigma Catalog No. 319511, or similar)
- 1.0N Hydrochloric Acid (HCl) Solution (Sigma Catalog No. 318949, or similar)
- Nylon Filter with 0.22 µm Pore Size (Sigma Catalog no. Z290807, or similar)
- 12 mm Polycarbonate Cell Culture Plate Insert with 12 µm Pore Size (Millipore Catalog No. PIXP01250, or similar)
- 24 well, Non-Treated, Multiple Well Plates (Corning Catalog No. 3738, or similar)

1. Formulate Kreb's Buffer Stock Solution by combining 5.96 g HEPES powder, 6.72 g Sodium Chloride (NaCL), 2.02 g Sodium Bicarbonate ($NaHCO_3$), 0.3728 g Potassium Chloride (KCl), 0.2033 Magnesium Chloride Hexahydrate ($MgCl_2$-$6H_2O$), and 1.0 g Bovine Serum Albumin (BSA) in a 1 L volumetric flask. Fill flask to a final volume of 1 L with sterile water and stir until components are dissolved.
2. Add 0.3675 g Calcium Chloride Dihydrate ($CaCl_2$-$2H_2O$) to Kreb's Buffer Stock Solution and stir until component is dissolved.

NOTE: Calcium Chloride Dihydrate ($CaCl_2$-$2H_2O$) may not dissolve completely until pH is adjusted.

3. Adjust the pH of the Kreb's Buffer Stock Solution to 7.3-7.5 by adding either 1.0N Sodium Hydroxide (NaOH) or 1.0N Hydrochloric Acid (HCl) as necessary.

Table 2. Kreb's Buffer Stock Solution Formulation

Component	Amount Added	Final Concentration
HEPES powder	5.96 g/L	25 mM
Sodium Chloride (NaCL)	6.72 g/L	115 mM
Sodium Bicarbonate ($NaHCO_3$)	2.02 g/L	24 mM
Potassium Chloride (KCl)	0.3728 g/L	5mM
Magnesium Chloride Hexahydrate ($MgCl_2$-$6H_2O$)	0.2033	1 mM
Calcium Chloride Dihydrate ($CaCl_2$-$2H_2O$)	0.3675 g/L	2.5 mM
Bovine Serum Albumin (BSA	1.0 g/L	0.1%

4. Filter the Kreb's Buffer Stock Solution through a nylon filter with 0.22 µm pore size into a sterile 1 L bottle. Store Kreb's Buffer Stock Solution at 2℃-8℃ for up to 12 weeks.
5. Formulate 280 mM Glucose Solution by dissolving 2.5 g D-(+)-Glucose (Dextrose) in 10 mL Kreb's Buffer Stock Solution in a sterile 50 mL conical tube. Thoroughly mix the solution, then add Kreb's Buffer Stock Solution to a final volume of 50 ml. Filter the combined solution through a nylon filter with 0.22 µm pore size into a sterile 50 mL conical tube. Store 280 mM Glucose Solution at 2℃-8℃ for up to four weeks.
6. Formulate High Glucose (28 mM) Solution by combining in 5 mL 280 mM Glucose Solution and 45 mL Kreb's Buffer Stock Solution in a sterile 50 mL conical tube. Thoroughly mix the solution, and use within 24 hours.
7. Formulate Low Glucose (2.8 mM) Solution by combining in 5 mL High Glucose (28 mM) Solution and 45 mL Kreb's Buffer Stock Solution in a sterile 50 mL conical tube. Thoroughly mix the solution, and use within 24 hours.
8. Remove the 400 IEQ of islet suspension from the original culture using a ***wide orifice pipette tip*** and transfer to a 100 mm petri dish. Fill petri dish to a final volume of 10 mL with culture media. Please see Section VIII (Counting Islets & Determining Purity Using Dithizone, Page 4) for more information on counting islets. Place the dish into a 37°C humidified incubator with 5% CO_2 while preparing assay plate.

NOTE: If original culture contains less than 40 IEQ per mL ,the concentration of islets may be increased by securely placing the islet shipping vessel at ~20° angle, allowing the i slets to settle for 3-5 minutes and slowly removing media. Centrifugation should not be performed to concentrate islets.

9. Prepare the assay plate by adding 1,000 µL Low Glucose (2.8 mM) Solution to the well(s) in Row A and Row B of a 24 well, non-treated, multiple well plate. Add 1,300 µL Low Glucose (2.8 mM) Solution to the well(s) in Row C of the same 24 well, non-treated, multiple well plate. Add 1,300 µL High Glucose (28 mM) Solution to the well(s) in Row D of the same 24 well, non-treated, multiple well plate.

Figure 1. Plate Preparation

	1	2	3	4	5	6
A	1,000 µL [2.8 mM] Wash	1,000 µL [2.8 mM] Wash	1,000 µL [2.8 mM] Wash	(blank)	(blank)	(blank)
B	1,000 µL [2.8 mM] Equilibrate /Rest	1,000 µL [2.8 mM] Equilibrate /Rest	1,000 µL [2.8 mM] Equilibrate /Rest	(blank)	(blank)	(blank)
C	1,300 µL [2.8 mM] Low Gluc. Test	1,300 µL [2.8 mM] Low Gluc. Test	1,300 µL [2.8 mM] Low Gluc. Test	(blank)	(blank)	(blank)
D	1,300 µL [28 mM] High Gluc. Test	1,300 µL [28 mM] High Gluc. Test	1,300 µL [28 mM] High Gluc. Test	(blank)	(blank)	(blank)

10. Using sterile forceps, place 12 mm polycarbonate cell culture plate insert(s) with 12 µm pore size into the well(s) in Row A.
11. Cover the plate and place into a 37°C humidified incubator with 5% CO_2 for and allow media to equilibrate for one hour prior to use.
12. Remove the dish containing the islets from the incubator and transfer the islets to a sterile 1.5 mL centrifuge tube by centering the islets in the dish and removing the islets in 200 µL of media using a ***wide orifice pipette tip***. After the first collection, re-center any remaining islets in the dish and remove the remaining islets in 200 µL of media using a ***wide orifice pipette tip*** to yield 400 IEQ in 400 µL of culture media in the single, sterile 1.5 mL centrifuge tube.

NOTE: For statistical accuracy and relevance, it is suggested that the following protocol be performed in triplicate and the results averaged.

13. Remove the assay plate from the incubator. Using sterile forceps, remove the polycarbonate

cell culture plate insert(s) from the well(s) in Row A and place onto laboratory use, lint-free cleaning tissue.

14. Prepare islet suspension by gently, but thoroughly mixing, the suspension by inverting the suspension 2-3 times. Do not swirl.

NOTE: Fresh Human Pancreatic Islets may settle quickly. Care must be taken to ensure a representative sample is taken.

15. Using a ***wide orifice pipette tip***, slowly pipette 100 µL of well-mixed islet suspension from the 1.5 mL centrifuge tube into each polycarbonate cell culture plate insert. Allow the culture media to drain completely.
16. Wash the insert(s) by placing the insert(s) into the respective well in Row A. Allow the medium to cover the islets, and then lift the insert(s) allowing the medium to drain back into the well. Wash the insert(s) a second time. After the medium has drained after the second wash, place the insert(s) onto new, laboratory use, lint-free cleaning tissue to drain out liquid completely.
17. Equilibrate the insert(s) by placing the insert(s) into the respective well in Row B. Allow the medium to cover the islets. Cover the plate and place into a 37°C humidified incubator with 5% CO_2 for one hour.
18. Remove the assay plate from the incubator. Using sterile forceps remove the insert(s) from the well(s) in Row B, allowing the medium to drain back into the well in Row B. After the medium has drained, place the insert(s) onto new, laboratory use, lint-free cleaning tissue to drain out liquid completely.
19. Begin the Low Glucose Stimulation by placing the insert(s) into the respective well in Row C. Allow the level of media to equilibrate between the inside and outside of each insert.
20. Using sterile forceps, immediately remove the insert(s) from the well(s) in Row C, allowing the medium to drain back into the well in Row C. **Do not blot the insert on laboratory use, lint-free cleaning tissue.** Remove 300 µL of medium from each well in Row C and transfer medium to an appropriately labeled centrifuge which includes “LG0” (Low Glucose Zero Time) and well number (i.e. C1, C2, C3, etc.). As soon as the sample is collected, immediately replace the insert in its corresponding well in Row C. Samples can be stored according to ELISA manufacturer’s instructions.
21. Immediately after all Low Glucose Zero Time (LG0) samples have been collected, cover the plate and place into a 37°C humidified incubator with 5% CO_2 for one hour.
22. Remove the assay plate from the incubator. Using sterile forceps, remove the insert(s) from the well(s) in Row C, allowing the medium to drain back into the well in Row C. After the medium has drained, place the insert(s) onto new, laboratory use, lint-free cleaning tissue to drain out liquid completely. Allow the samples to rest by placing the insert(s) into the respective well in Row B.
23. While islets are resting in Row B, remove 300 µL of medium from each well in Row C and transfer medium to an appropriately labeled centrifuge which includes “LGS” (Low Glucose Stimulated) and well number (i.e. C1, C2, C3, etc.).
24. Using sterile forceps, remove the insert(s) from the well(s) in Row B, allowing the medium to drain back into the well in Row B. After the medium has drained, place the insert(s) onto new, laboratory use, lint-free cleaning tissue to drain out liquid completely.
25. Begin the High Glucose Stimulation by placing the insert(s) into the respective well in Row D. Allow the level of media to equilibrate between the inside and outside of each insert.
26. Using sterile forceps, immediately remove the insert(s) from the well(s) in Row D, allowing the medium to drain back into the well in Row C. **Do not blot the insert on laboratory use, lint-free cleaning tissue.** Remove 300 µL of medium from each well in Row D and transfer medium to an appropriately labeled centrifuge which includes “HG0” (High Glucose Zero Time) and well number (i.e. D1, D2, D3, etc.). As soon as the sample is collected, immediately replace the insert in its corresponding well in Row D.
27. Immediately after all High Glucose Zero Time (HG0) samples have been collected, cover the plate and place into a 37°C humidified incubator with 5% CO_2 for one hour.
28. Remove the assay plate from the incubator. Using sterile forceps remove the insert(s) from the well(s) in Row D, allowing the medium to drain back into the well in Row D. After the medium has drained, place the insert(s) onto new, laboratory use, lint-free cleaning tissue to

drain out liquid completely. Allow the samples to rest by placing the insert(s) into the respective well in Row B.

29. While islets are resting in Row B, remove 300 µL of medium from each well in Row D and transfer medium to an appropriately labeled centrifuge which includes “HGS” (High Glucose Stimulated) and well number (i.e. D1, D2, D3, etc.).
30. Store all collected samples at 2℃-8℃ if assayed within 24 hours or store at -20℃ +/- 5℃ if assayed within two weeks.
31. Analyze the actual insulin concentration (mg/ml) of each sample by using an appropriate ELISA kit (for example, Mercodia Insulin ELISA, Mercodia Catalog. No. 10-1113-01 or similar).
32. Calculate the average actual insulin concentration for each condition (Low Glucose Time Zero [LG0], Low Glucose Stimulated [LGS], High Glucose Time Zero [HG0], and High Glucose Stimulated [HGS]).
33. Determine the Stimulation Index (SI) by using the following equation:

$$\text{Stimulation Index (SI)} = \frac{([\text{HGS}] - [\text{HG0}])}{([\text{LGS}] - [\text{LG0}])}$$

X. Additional Available Testing (Additional Charge)

For some applications, additional testing results may provide information that may be difficult or time-consuming to obtain. The following test is not standardly performed on Lonza’s pancreatic islets, however, it is available for an additional charge. Please note that additional testing is initiated on the day of cell shipment. If you require additional testing, please specify at the time of order.

Glucose Stimulated Insulin Response (GSIR):

Test	Typical Results (F.I.O.)	Estimated Testing Time
Glucose Stimulated Insulin Response	2.716 +/- 0.93	4 days

Glucose Stimulated Insulin Response (GSIR) is calculated as the amount of insulin released at high glucose concentrations versus low glucose concentrations. After culturing at 37℃ for 12-24 hours, an aliquot of islets seeded onto transwell inserts and plated in low glucose (2.8 mM) for one hour at 37℃ to equilibrate sentence is a little strange. The inserts are then transferred to a second set of wells at low glucose for one hour at 37℃. Finally, the inserts are transferred to a final set of wells at high glucose (28 mM) for one hour at 37℃. Supernatants from the low and high glucose wells are collected and the amount of insulin released is determined by ELISA. The stimulation index is calculated as the amount of insulin released at high glucose versus low glucose and the average stimulation index of multiple aliquots is reported. Please see Section IX (Determining Glucose Stimulated Insulin Response, Page 5) for more information on performing this assay.

XI. Ordering Information

Pancreatic Islets can be ordered by visiting **www.lonza.com/islets** and completing the online order instructions.